adequate B cell function and are still dependent on IVIG infusions. All 5 patients with absence of B cell function had less than 25% B cell donor engraftment and 3 of them had received ablative conditioning. Less than 25% donor B cell engraftment correlated with abscense of function (P = .006). 4/5 patients with absent B cell function have IL2RG genetic defect (OR=5.12, P = .038). All the recipients of MMUCB achieved faster and more reliable B cell function compared to other donor sources (P = .003). Hence absence of adequate B cell function is most closely associated with pts who have IL2RG defect irrespective of conditioning. In addition, donor source influence the speed and probably the ultimate success of B cell engraftment (MMUCB>haplo donors).

186

Assessment of the Effect of Nilotinib (Tasigna) Maintenance Therapy After Allogeneic Stem Cell Transplantation in Patients with Advanced CML and Ph+ ALL On Immune Reconstitution and Lymphocyte Function

Nira Varda-Bloom [1], Raz Somech [2], Yulia Volchek [3], Jacqueline Davidson [1], Atar Lev [1], Ronit Yerushalmi [3], Avichai Shimoni [3], Arnon Nagler [3]. [1] Division of Hematology, Chaim Sheba Medical Center, Ramat Gan, Israel; [2] Safra Children's Hospital, Chaim Sheba Medical Center, Ramat Gan, Israel; [3] Division of Hematology and Bone Marrow Transplantation, Chaim Sheba Medical Center, Tel-Hashomer, Israel

TKIs are known to affect T and NK cells. We therefore assessed immunological reconstitution and function in 24 pts with advanced CML and Ph+ ALL who received Nicotinib as maintenance therapy post alloSCT (study CAMN107AIL03T). At 6 months after alloSCT, 11 of 15 pts with advanced CML had attained CCyR, 11 of 15 pts with advanced CML had attained a MMR or better, and 5 of 7 pts with Ph+ ALL attained a CR. The median OS was 16 months, with predicted 1- and 2- year rates of 55% (95% CI, 32% – 72%) and 50% (95% CI, 28% – 68%), respectively. The median PFS was 11 months, with predicted 1- and 2- year rates of 50% (95% CI, 28% – 68%) and 38% (95% CI, 17% – 59%), respectively. Immunological testing was performed pre- and post Nilotinib maintenance therapy in 12 pts in this study. The relative percentage of T- lymphocyte subsets (assessed by FACS) and total lymphocytes number were stable during Nilotinib maintenance therapy, while a 7.8±2.5 fold increase in B cells was noted. T cell mitogenic response with αCD3 and PHA (stimulation index ratio) was sustained (2.5±1.0, vs. 2.8±1.05 and 3.3±1.3 vs. 5.3±2.9 stimulation. Mean thymic output determined by TREC quantification pre-, during and post Nilotinib administration was 81.8±108, 81.2±90.3 and 142.8±197.4 copies per 0.5ug DNA indicating continuous thymopoiesis. Similarly, no significant change of the TCR repertoire was observed during Nilotinib treatment. Normal expression of the TCR repertoire was detected in 15.1±5.5 and 15.3±5.6 of the examined TCRs. NK cytotoxic activity against K562 expressed as fold of change from baseline, also remained stable during Nilotinib treatment (2.8±1.1 and 2.3±0.8, respectively). In summary, Nilotinib maintenance therapy post alloSCT in pts with advanced CML and Ph+ALL did not interfere or jeopardized immune reconstitution and function.

187

Evolution of T Cell Repertoire Diversity After Reduced-Intensity Conditioning and Double Umbilical Cord Blood Transplantation with or without Exposure to FT1050 (16,16-dimethyl Prostaglandin E2)

Sarah Nikiforow [1], Sean M. McDonough [1], Ryan O. Emerson [2], David Hamm [2], Haesook Kim [3], Karen Ballen [4], Vassiliki A. Boussiotis [5], Robert J. Soiffer [6], Joseph H. Antin [1], Jerome Ritz [6], Corey Cutler [1]. [1] Hematologic Malignancies, Dana-Farber Cancer Institute, Boston, MA; [2] Adaptive Biotechnologies, Seattle, WA; [3] Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Boston, MA; [4] Massachusetts General Hospital, Boston, MA; [5] Beth Israel Deaconess Medical Center, Boston, MA; [6] Dana-Farber Cancer Institute, Boston, MA

In double umbilical cord blood (UCB) transplantation (dUCBT), *ex vivo* modulation of a single UCB unit with 16, 16 dimethyl prostaglandin E_2 ($dmPGE_2$) accelerates neutrophil engraftment and leads to preferential hematopoietic dominance with complete T cell chimerism as early as 13 days from dUCBT. We sought to determine whether $dmPGE_2$ affected the recovery of T cell diversity after dUCBT.

Methods: dUCBT recipients received fludarabine, melphalan, and ATG conditioning with Tacrolimus and Sirolimus GvHD prophylaxis. TCRb CDR3 regions were amplified and sequenced using the ImmunoSeq assay (Adaptive Biotechnologies) from a median of 171 ng of T cell gDNA (range 0.8 - 400.0 ng) at 3 and 12 months from dUCBT. We analyzed 5 paired samples with $dmPGE_2$-dominance (PD); 3 paired and 7 single 12 month untreated (U) samples; and 5 paired and 1 single 12 month $dmPGE_2$ treated sample with untreated-dominance (UD). The U+UD groups were combined as one control cohort.

Results: PD $CD3^+$ cells were lower at 3 months versus the U+UD group (P = .057, Table) but this trend diminished by 12 months (P = .11). Lower $CD3^+$ counts were reflected in lower total TCR alpha diversity in PD samples at both time points (3 month median 585 vs. 3905 unique TCR sequences, P = .079;

Table

	3 Months after dUCBT				12 Months after dUCBT			
	$dmPGE_2$-dominant	Untreated/ Untreated-dominant	*P* value - T test	*P* value - U test	$dmPGE_2$-dominant	Untreated/ Untreated-dominant	*P* value - T test	*P* value - U test
WBC	5900.0	4850.0	0.377	1	4300.0	6500.0	0.049	0.035
$CD3^+$ count	10.6	50.0	0.097	0.26	159.4	444.2	0.016	0.014
% $CD3^+$ of lymphocytes	2.55%	11.00%	0.066	0.057	12.30%	31.85%	0.161	0.11
Richness Alpha Diversity	585	3905		0.079	2729	12062.5		0.22
Clonality 1-Normalized Entropy	0.1381	0.2454	0.090	0.19	0.1105	0.2326	0.945	0.54
Total Repertoire Overlap Index					0.0873	0.3884	0.027	0.057
Persistence of Top 10 Clones					2	10		0.040

12 month median 2729 vs. 12062.5 unique TCR sequences, $P = .22$). Median lymphocyte counts did not increase between 3 and 12 months in the PD group as in the U+UD group, but the proportion of $CD3^+$ cells within lymphocytes increased in both groups. PD T cell repertoires were less clonal than U+UD samples at 3 months (1-normalized entropy: 0.14 vs. 0.25, $P = .09$) and 12 months (1-normalized entropy: 0.11 vs. 0.23, $P = .95$), with more even frequency distribution of TCRs. PD T cell repertoire was more dynamic than the U+UD controls with a significantly decreased T cell clonal persistence between 3 and 12 months (TCR sequence overlap: 8.73% vs. 38.84%, $P = .027$).

Conclusions: *Ex vivo* $dmPGE_2$ modulation with $dmPGE_2$ dominance results in delayed T cell and lymphocyte recovery, but less clonality despite a T cell repertoire restricted by low $CD3^+$ counts. The reduced oligoclonality and rapid T cell turnover may indicate enhanced thymopoiesis. Correlation with TRECs and clinical infectious outcomes is ongoing.

188

Monitoring CMV-Specific CD8+ T-Cell Responses After Allogeneic Stem Cell Transplantation: A New Way of Guiding Anti-Viral Therapy

Amaya Zabalza [1,2], Miriam Ciaurriz [2], Lorea Beloki [2], Natalia Ramirez [2], Ana Gorosquieta [1], Mercedes Rodriguez Calvillo [1], Eva Bandres [1], Cristina Mansilla [2], Eduardo Olavarria [1,2]. [1] Complejo Hospitalario de Navarra; [2] Biomedical Research Center of Navarra

CMV reactivation after allo-SCT is usually monitored by CMV-PCR. We present the results of a pilot study looking at the additional use of Multimers for monitoring CMV-specific CD8+ T-cells at different intervals after allo-SCT.

10 patients (4 males) that underwent allo-SCT from HLA-identical siblings (n=4) or HLA-matched unrelated (n=6) donors between May 2010 and May 2012 were enrolled. The diagnosis was acute leukemia (n=6), lymphoproliferative disorders (n=2) and myelodysplastic syndromes (n=2). All patients and 8 donors were CMV seropositive. All patients were positive for the HLA-A*0201 allele. Pentamers (Proimmune, UK) and Streptamers (Gottingen GmbH, Germany) were directed against the epitope NLVPMVATV of the $pp65_{495\text{-}503}$ protein of CMV in the context of HLA-A*0201. Samples were obtained from engraftment at 30-day intervals until day +180 and 2-monthly thereafter.

The median follow-up was 12.6 (4-21) months after SCT. Engraftment occurred in all patients at a median of 21.2 (15-27) days. Acute GVHD was diagnosed in 4 patients at a median of 63.3 (25-92) days. One patient developed acute GVHD following infusion of donor lymphocytes for the treatment of mixed chimerism on day +323.

Three patterns were observed. In 2 patients no CMV-specific CD8+ T-cells could be detected after SCT despite several episodes of CMV-PCR reactivation requiring prolonged antiviral treatment. In 5 patients CMV-PCR reactivation triggered a rapid increase of CMV-specific CD8+ T-cells (median 112.2 x 10^5/L, range 1.3-279.7 x 10^5/L). However, the CMV-PCR became immediately negative and antiviral treatment was stopped in all reactivations within 2 weeks. Subsequent CMV-PCR reactivations also lasted under 2 weeks in this group. Finally, 3 patients showed an early immune reconstitution with CMV-specific CD8+ T-cells detected (median 1.3 x 10^5/L, range 0.3-2.3 x 10^5/L) at a median of 21 (17-33) days post-SCT. No CMV-PCR reactivation was observed in this group.

We conclude that monitoring CMV-specific T-cell immunity after allo-SCT in combination with CMV-PCR may be able to distinguish patients at higher risk of CMV reactivation and in need of prolonged antiviral therapy. Patients with CMV-specific CD8+ T-cells detectable at the time of CMV-PCR reactivation may only need a short course of antiviral therapy, while those with early and persistent CMV-specific CD8+ T-cells may be at a very low risk of developing CMV disease in the long-term.

189

Elevated Gamma Delta T Cell Recovery Following Hematopoietic Stem Cell Transplantation Associated with Improved Long Term Overall Survival in Pediatric Patients with Acute Leukemia

Ross Perko [1], Paul Thomas [2], Guolian Kang [3], Mari Hashitate Dallas [4]. [1] Hematology and Oncology, St. Jude Children's Research Hospital, Memphis, TN; [2] St. Jude Children's Research Hospital, Memphis, TN; [3] St. Jude Children's Research Hospital, Memphis, TN; [4] Bone Marrow Transplantation and Cellular Therapy, St Jude Children's Research Hospital, Memphis, TN

Recent studies show that accelerated γδ T cell reconstitution after hematopoietic stem cell transplantation (HSCT) is associated with improved overall survival (OS) though the mechanisms have not been elucidated. We evaluated 102 consecutive pediatric patients with acute leukemia undergoing HSCT at St. Jude Children's Research Hospital from 1996-2011. The median age of the patients was 10.5 ± 5.9 yrs. (range, 0.6-25.2) and median follow up was 2.7 ± 1.8 yrs. (range 0.2-6.0). There were 57% males, 43% females and 59% with ALL and 41% with AML. There were 14 patients with elevated γδ T cells ($\geq 1.75 \times 10^5$ cells/ml) and 88 with low/normal γδ T cells ($< 1.75 \times 10^5$ cells/ml). There were no significant differences between the two groups with respect to age, sex, disease or donor source, p=0.7, 0.5, 1 and 0.07 respectively. Fours years after HSCT, Overall Survival (OS) was significantly higher for patients in the elevated group compared to the patients in the low/normal group, 93% and 60%, respectively, p=0.0173. Survival without relapse or graft failure, Event Free Survival (EFS), was significantly higher in the elevated group compared to the low/normal group, 85.7% and 58.0%, respectively. Since T cell reconstitution following HSCT is age dependent, we determined if γδ T cell recovery correlated with age and/or $CD3^+$ cells. Multivariate analysis showed no correlation between the number of $CD3^+$ and γδ T cells. In fact, 13 of 14 patients that recovered with increased number of γδ T cells had normal or low numbers of $CD3^+$ cells. Thus, γδ T cell recovery is not a simple correlate of T cell reconstitution. Because γδ T cells play a central role in maintaining intestinal epithelium integrity, we evaluated the incidence of gut GVHD. We found a significant lower rate of gut GVHD in the elevated group compared to the low/normal group, 0% and 17% respectively. Furthermore, the number of γδ T cells in patients with cGVHD (2.3 x10^5 cells/ml) was significantly lower compared to patients without cGVHD (6.2 x10^5 cells/ml), p=0.01. This suggests that γδ T cell may protect against gut and cGVHD. Since accumulating evidence suggests that γδ T cells contribute to both innate and adaptive immune responses during infections, we evaluated the rate and types of infection between the two groups. We found a significant lower incidence of infections reported in the elevated group compared to the low/normal group, 21% and 54% respectively p=0.02. Furthermore, the elevated group had only viral infections while the low/normal group had viral, bacterial and fungal infections. In summary, this is the first reported study of γδ T cell recovery after HSCT in pediatric patients and adds new insights into the role γδ T